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Evaluation of Sucralose as a Sugar Substitute in Different Food Matrices of the Sri Lankan Market Using a Validated HPLC Method

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Abstract

Excessive consumption of added sugars is a major contributor to diabetes and other non-communicable diseases, driving the global demand for low-sugar or sugar-free food products. Sucralose, a non-nutritive sweetener with high stability and sugar-like taste, is widely used in food, beverages, pharmaceuticals, and dietary supplements. It has been recognized as "Generally Recognized as Safe" (GRAS) by the U.S. Food and Drug Administration. However, its use must comply with national and international food safety regulations to ensure that acceptable daily intake (ADI) and maximum permitted levels are not exceeded. This study aimed to develop and validate a simple and reliable High-Performance Liquid Chromatography (HPLC) method for the quantification of sucralose in food and beverage samples available in the Sri Lankan market. Separation was achieved on a Hi-plex-H column (300 × 7.7 mm, 8 µm particle size) at 350C using ultra-pure water as the mobile phase were monitored using refractive index detector. Performance of the developed method was assessed according to the Eurochem guidelines. The method demonstrated a limit of detection (LOD) and quantification (LOQ) of 1.2 mg/kg and 2.0 mg/kg, respectively, with a linear range of 1–600 mg/kg (R^2 = 0.9997). Recovery ranged from 81.2% to 108.0%, with precision (%RSD) below 6.1% for repeatability and reproducibility. The expanded measurement uncertainty (k = 2) was 7.7%. The validated method is accurate, precise, and suitable for routine determination of sucralose in food and beverage matrices.

Keywords: Sucralose, Non-nutritive sweeteners, HPLC, Method validation, Food analysis

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1 Introduction

The World Health Organization (WHO) has promoted lowering sugar consumption to less than 5% of total Energy intake in the form of free sugars. [1-2] Many countries have taken steps to

regulate the level of added sugar in their popular food items. Even in developing countries, market demand for food and beverage products with alternative nutritional characteristics such as zero-caloric or sugar-free food and soft drinks to fulfill the low sugar requirements of the consumer to maintain good health [3]. Presently, consumer health awareness has increased in beverages and their commercial preparations and the demand for food products that sustain better health with a greater variety of low-calorie products [4]. The Health Ministry of Sri Lanka has introduced a traffic light system for food and drinks, enabling consumers to identify them as low, medium, or high added sugar. However, these regulatory influences and the awareness among the public of the hazardous health effects of high sugar consumption have forced food manufacturers to come up with low-sugar products [5 -7]. As a result, there is a growing trend of using non-caloric, high-intense sweeteners as sugar substitutes in food products, both carbonated and non-carbonated beverages, and sweeteners in order to maintain the sweetness while reducing calorie content. The use of sugar substitutes, both nutritive and non-nutritive, has become increasingly prevalent in the food industry worldwide due to rising health concerns related to excessive sugar consumption, particularly in addressing issues such as obesity, diabetes, and cardiovascular diseases. In Sri Lanka, as in many other countries, there has been a growing demand for alternative sweeteners in food products to cater to health-conscious consumers. [7] This has led to the introduction of a variety of sugar substitutes that include both nutritive sweeteners, such as sugar alcohols like sorbitol, mannitol, and maltitol. and non-nutritive sweeteners such as aspartame, acesulfame K, stevia, sucralose, and saccharin. When considering food safety and quality, it is essential to ensure legislated limits within the standard by verifying the presence and concentration of sweeteners [8-9].However, the regulatory compliance of both nutritive and non-nutritive sweeteners, whether natural or artificial, changes across different countries and regions. These regulations are intended to ensure consumer safety, maintain fair market practice, and support local food manufacturers to align with national and international food safety standards [8-10]. Therefore, to facilitate consumer protection, ensure that the composition of food products is accurately labeled and that potentially harmful substances are not used beyond acceptable limits. In this regard, given the diverse range of sweeteners used, the need for reliable, accurate, and comprehensive analytical methods for their identification and quantification has become critical in ensuring product safety, quality, and regulatory compliance in the food industry. These analytical methods must be capable of detecting and differentiating between both nutritive and non-nutritive sweeteners, fulfilling the goals of establishing multi-compound analytical methods that can quantify a wide range of nutritive and non-nutritive sweeteners present in complex food matrices [8-10]. Sucralose is an artificial sweetener derived from sucrose through a modification process. Its chemical structure differs from sucrose due to the replacement of three hydroxyl groups with chlorine atoms. Specifically, sucralose's molecular formula is 1,6-dichloro-1,6-dideoxy-β-d-fructofuranosyl-4-chloro-4-deoxy-α-d-galactopyranoside, which describes its two sugar units fructose and glucose modified with chlorine at key positions.

FDA approved sucralose for use in 15 food and beverage categories, which included a wide range of products such as beverages, frozen dairy desserts, baked goods, and sugar substitutes like Splenda. In 1999, this approval was expanded to allow sucralose to be used as a general-purpose sweetener in all food and beverage categories. European Union, approved in 2004 as a food additive E 955, for use in a variety of products, including desserts, alcoholic beverages, spreads, cereals, and chewing gum.Sucralose is synthesized by substituting three chlorine atoms into the sucrose molecule through a five-step chemical process. This selective chlorination strengthens the

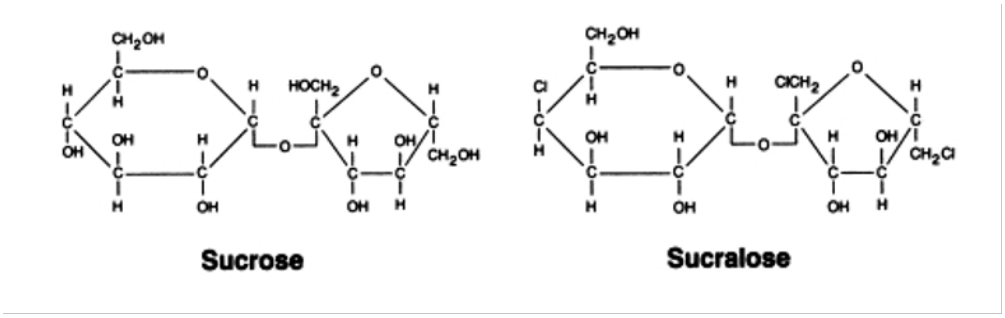

Figure 1: Molecular structures of sucrose and sucralose

molecule's stability, making it resistant to breakdown by acids, bases, and enzymes that typically break down sugars. Further, due to the presence of three chlorine atoms, owing to the retention of five hydroxyl groups in its structure of sucralose facilitates its solubility in water [11]. Sucralose is approximately 600 times sweeter than sucrose, offering a similar taste profile without the calories typically associated with sugar. Because sucralose is not metabolized by the human body, it has no caloric value, making it a popular choice as a non-nutritive sweetener. Sucralose, an organochlorine synthetic sweetener, has been the subject of various health and biological concerns despite its widespread use in food and beverages regarding its health profile.Sucralose is not metabolized by the human body and is excreted unchanged in the urine. However, the presence of chlorine atoms in its chemical structure raises questions about its potential for toxicity and bioaccumulation. Some animal studies have indicated potential effects on liver and kidney function, though human studies have shown little evidence of such concerns at typical consumption levels. Although extensive research has been conducted, there has been concern over the long-term consumption of sucralose and its potential link to cancer. The U.S. FDA, EFSA and other health agencies have concluded that sucralose is safe for human consumption at recommended levels, and no carcinogenic effects have been definitively shown in long-term studies. However, ongoing debates and studies are focusing on its safety over extended periods.Sucralose is highly stable and resistant to degradation in the environment. Studies have shown that it can persist in wastewater and aquatic environments, raising concerns about its ecological impact and potential accumulation in ecosystems.

1.1 Health effects of sweetener sucralose

Some animal studies have raised questions about the impact of artificial sweeteners like sucralose on the nervous system, including potential neurotoxic effects. However, conclusive evidence from human studies remains lacking, and the safety of sucralose from a neurological perspective is still debated [12]. These concerns have led to calls for further research to fully understand the long-term biological and health effects of sucralose consumption, particularly in large quantities or over extended periods. Despite these issues, regulatory agencies continue to deem sucralose safe at established ADI levels, and it remains one of the most widely used artificial sweeteners globally. Although sucralose is used globally in reduced-calorie and diet foods and beverages, issues regarding its biological effects and, therefore, its health profile have raised concerns.

Currently in Sri Lanka, most of the nonalcoholic beverages, food supplements, and tabletop sweeteners are found to be sweetened with sucralose. However, according to the Food Act, Sucralose has been regulated with the maximum level of permissible in various food groups. Hence,

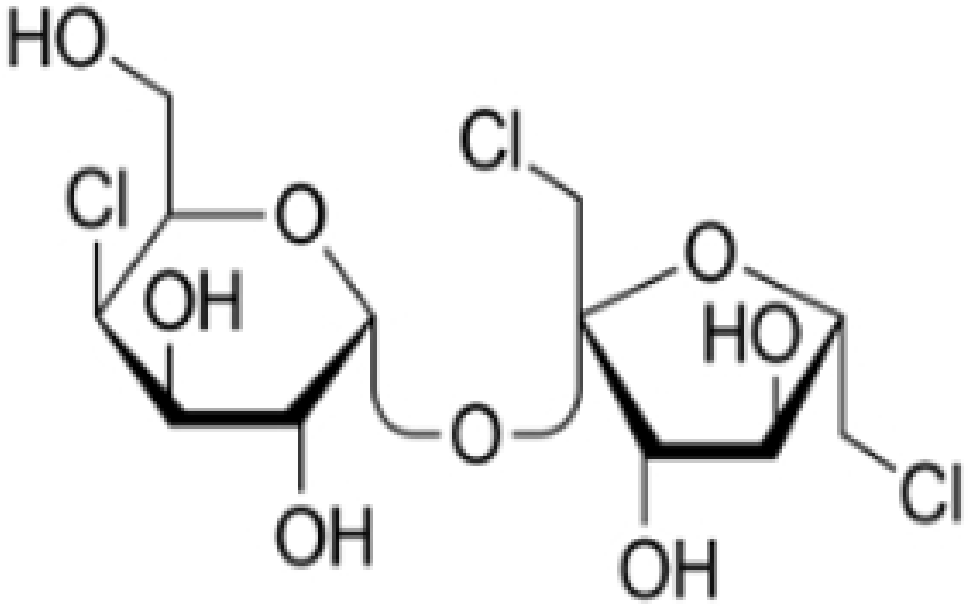

Figure 2: Structure of sucralose

the identification and quantification of sucralose-containing products are important to ensure consistency in products to make healthier food choices. [8,13]

2 Methodalogy

Development of sucralose method

Three different HPLC methods were evaluated for sucralose analysis, including using an amino column with an RI detector and Hiplex-H column with an RI detector. The HPLC-RID system with the Hiplex –H (300 x 7.7 mm, 8 µm particle size) column using de-ionized water was found to be the most economical and environmentally friendly supported and comparatively précised method. Then verification was done for the robustness, that is to emphasize the ability of a method to stand deliberate discrepancies in the operating parameters and there by ensure the performance of the method through its normal usage. This was confirmed that with a slight change of either flow rate, column pressure and with temperature the method recovery was within the acceptance range and as such the method was validated for the parameters of selectivity, accuracy, precision, intermediate precision, linearity, working range, LOD and LOQ and uncertainty according to the international method validation guidelines [14,15].

Stock standard solution of sucralose preparation

Stock standard solution of 1000 ppm was prepared in ultra-pure water obtained from Milli Q water system and it was diluted to prepare the appropriate calibration standard solutions and the solvent system which was used as the mobile phase contained only ultra-pure water.

Sample preparation for sucralose analysis

All samples with carbonated were degassed by sonication for 10 minutes and instant drinks were prepared according to the manufacturer's directions. Then the samples were diluted 10 times with ultra-pure water. Homogeneous 1.0 g of solid and semi solid sample portions or 1.0 mL of liquid samples were brought up to 10.0 mL volumetric flask using ultra-pure water. The mixture was then extracted by using vortex for four minutes and followed by centrifugation at 10000 rpm for 8 minutes only for semi or solid samples. Supernatant and liquid samples was then filtered via 0.45 micro porous film prior to HPLC analysis [16-17].

Analytical Procedure

The chromatographic separation was achieved using ultra-pure water as the mobile phase on Hiplex –H (300 x 7.7 mm, 8 µm particle size) operated at 35 o C at the flow rate of 1.0 mL / min for 12 minutes. And the sample injection volume was 10µL.

Method validation of developed sucralose method

Method validation was done as per the. as per the Eurachem guidelines. Accuracy, precision, linearity, working range, LOD, LOQ and recovery of the analytical technique were assessed for the validation [14-15].

3 Results and Discussion

Analysis of Sucralose

Method validation was done as per the. as per the international guidelines [14-15]. Accuracy, precision, linearity working range, limit of detection (LOD), Limit of quantification (LOQ) and recovery of the analytical technique were assessed for the validation. The chromatogram presenting the separation of sucralose is shown in Figure. 3.

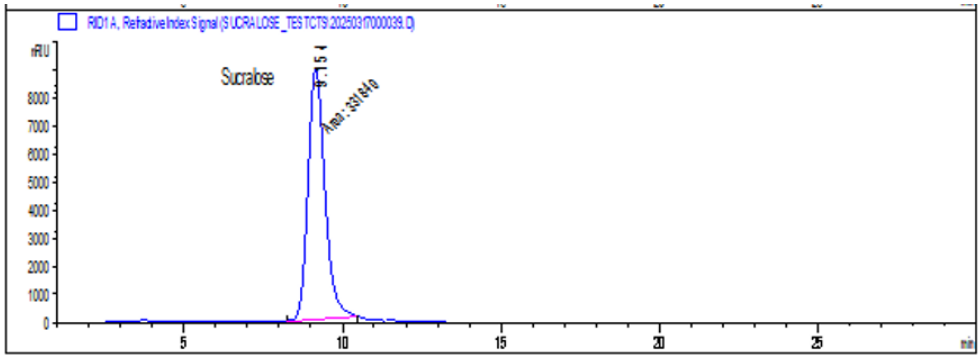

Figure 3: Chromatogram of Sucralose standard separation

Validation of the method performed in terms of LOD, LOQ, linearity, working range, repeatability, intermediate precision, as recovery using analytical standard. As described in the table 1,

LOD and LOQ values for sucralose was 1.2 mg/kg and 2 mg/kg respectively. And the obtained linear dynamic range was 1 to 600 mg/kg, having the regression coefficient of 0.9997, which shows that the method is capable of producing good sensitivity shown in figure 3.13. However, as per the method describe by Sang [18] fast and sensitive reverse phase -HPLC method with RID, was introduced with the working range of 20 400 mg/ L, and LOD of 2.4 mg/kg. However, the mobile phase of 40:60 was as methanol: 0.125% potassium hydro phosphate, was comparatively less environmental friendly and the LOD was slight higher than the method which was introduced that is 2.4 mg/kg. The method provides by Hanko [19] was reliable, sensitive, and efficient way to detect sucralose in table top sweeteners and beverage without sample preparation and providing with comparatively lower LOD of 0. 86 mg/kg, using high-performance anion-exchange (HPAE) chromatography coupled with pulsed amperometric detection (PAD) is more complex and cost effective. However, the method has not been validated for other matrixes like dairy and cereal based samples and the reporting value(2.61mg/kg) or limit of quantification is comparatively high. Further, another HPLC-ELSD method by Wang [20] showed good linearity in the range of 2-600 µg/mL with LOD range of 0.5-2.0mg/kg, good repeatability (RSD¡3%, n=6), but the and obtained recoveries were from 96.8% to 101.2%, and such, is more sensitive. As describe by Batchu [21], method developed based on SPE-LC-MS/MS for sucralose determination in the matrix of water was a reference technique for a alternative spectroscopic method with the 0.002mg/kg from the highest level of the range of LOD. And the method can be used successfully to identify the residual quantities in water in US as they regulated to consume less than approximately 5.0 mg per person per day. Therefore, this type of determination will be valuable for a future residual base study on drinking water in Sri Lanka. However, most of, these methods often require costly equipment and comprise with complex procedures with more time-consuming, which limits their suitability and practicality in routine analysis. Therefore, developing accessible detection technique with overall efficiency and cost-effectiveness of sucralose analysis methodology is crucial for ensuring food safety, public health monitoring, and environmental risk assessment, especially as concerns about the environmental impact and potential health risks across a wide range of food, pharmaceutical, and environmental. will play a key role in advancing scientific knowledge and addressing public and regulatory concerns regarding sucralose.

Summery of Method Validation

Table 1: Method validation data of Sucralose analysis

Method Validation Parameters of Sucralose Determination	**Value**
Working range (mg/kg)	1.2–600
Linearity (Correlation coefficient)	0.9997
Limit of Detection (mg/kg)	1.2
Limit of Quantification (mg/kg)	2
Accuracy as spiked Recovery (%)	81.7–108.0
Precision – Repeatability (% RSD)	¡ 4.1
Intermediate precision (% RSD)	¡ 7.4
Uncertainty (k=2)	7.7

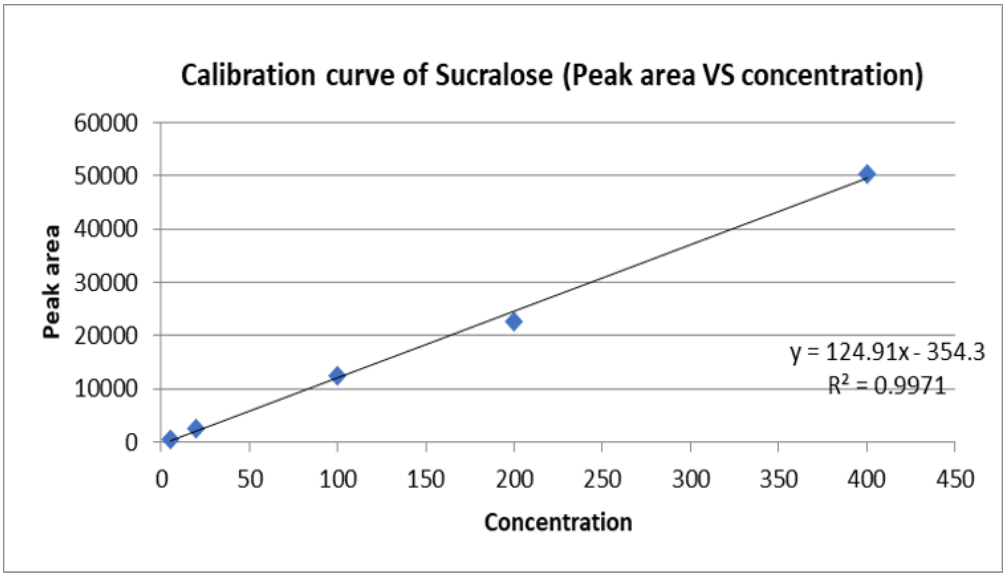

Figure 4: Calibration curve of Peak area VS Concentration for Sucralose

Available Sucralose content

The results from Table 2 of the sucralose content in non-alcoholic beverages found to be less than the regulated amount of 300 mg /kg and all other types of matrix were less than the maximum permissible level except chocolate flavored ice-cream which was classified as a milk-based and milk derivative-based preparation.

4 Conclusion

In this study, the optimized national standard method was used to detect sucralose in drink, which was stable in operation and could fully meet the needs of daily analysis in terms of precision, accuracy and detection limit, as well as compared with the national testing standards[22] and the results of the study of some scholars [9,23-24], the analysis speed is accelerated, the sensitivity is improved, the toxicity and cost are reduced by replacing Acetonitrile with Methanol, the stability is improved by adding phosphate , the balance of the detector is accelerated, the use of mobile phase is saved, the detection efficiency is improved, and the results of this study are consistent and suitable for daily testing of a large number of drink samples, which is consistent with those of Gan Bingbing [7] . And compared with the previous studies of Chen Xiaoxia [25], the column temperature and detection temperature are reduced, the loss of the column is reduced, and the service life of the column is extended. The author has also tried to use with the same water-based chromatographic of Wang Guihua [8]] column in the study, although ultra-pure water is used as the flow is easier to prepare, but the peak time is relatively late, resulting in the waste of reagent, and the water-based chromatographic column on the pretreatment method has relatively strict requirements, improper treatment, will cause damage to the water-based chromatographic column, reduce the service life of the column, should not be promoted. In the future, we can further investigate whether this research method can be applied to the detection of other types of samples, and continue to optimize the pre-treatment method to reduce the time needed for the

Table 2: Sucralose contents in different matrices available in Sri Lanka market

Matrix	Name of beverage	Sucralose content (mg/L)
Non-Carbonated beverages	Peach flavoured drink	349.8±0.93
	Apple flavoured drink	38.4±0.55
	Mixed fruit drink	67.5±0.0
	Mixed fruit brand 2	114.6±0.14
	Mango drink	107.8±3.30
	Pineapple drink	110.4±0.74
	Aloe-vera drink	68.9±0.22
Carbonated beverages	Ginger beer	45.7±0.28
	Sugar free Beverage 1	90.1±0.30
	Sugar free Beverage 2	17.2±0.00
	Energy beverage	149.1±0.5
	Fruity beverage	28.5±1.70
	Beverage 1	38.3±0.02
	Beverage 2	33.4±0.04
	Beverage 3	39.7±0.02
	Beverage 4	57.0±0.80
Table top sweetener	Sweetener 1	101.2±1.40
	Sweetener 2	85.7±0.42
	Sweetener tablet	14.4±1.50
Miscellaneous	Food supplement 1	112.2±0.65
	Food supplement 2	216.2±0.72
Dairy based	Vanilla Ice-cream	41.5±1.50
	Chocolate Ice-cream	516.2±2.00

whole experimental cycle and improve the detection efficiency

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